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Risk Assessment Research, 1992-1994:

An Overview of the USDA Biotechnology Risk Assessment Research Grants Program

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National Biological Impact Assessment Program

NBIAP is designed to facilitate and assess the safe application of new techniques for genetically modifying plants, animals, and microorganisms to benefit agriculture and the environment. The headquarters staff of NBIAP consists of **Dr. David MacKenzie**, **Dr. Ann Lichens-Park**, **Jay Blowers**, **Thelma Tennant**, and **Sally Ann Andraitis**. Questions about this program or the Biotechnology Risk Assessment Research Grants Program may be addressed to:

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Introduction

Agricultural biotechnology offers significant promise for providing greater amounts of more nutritious, appealing foods at less cost to the natural environment. However, the products of agricultural biotechnology may pose risks to ecosystems or human health, and the traditional risk assessment process, which was developed primarily to assess the hazards associated with chemicals, cannot be easily applied to agricultural biotechnology. Thus, it was necessary to develop new approaches to assess the possible risks associated with the development and deployment of agricultural biotechnology products.

In recognition of this need, in Section 1668 of the 1990 Farm Bill, Congress established a competitive grants program within the U.S. Department of Agriculture (USDA) to fund biotechnology risk assessment research. The Biotechnology Risk Assessment Research Grants Program is supported by a 1 percent set-aside of USDA funds allotted annually for biotechnology research. It is administered jointly by the Cooperative State Research, Education, and Extension Service and the Agricultural Research Service, with participation by the Forest Service.

The purpose of the program is to support science-based regulatory decisionmaking. Each year, eligible scientists submit research proposals, which are reviewed by scientific peer panels. Supplemental evaluations of proposals are sent in from external reviewers by mail.

Risk Characterization

Risk analysis is a two-step process encompassing risk assessment and risk management. Risk assessment is the science-based evaluation and interpretation of factual information in which a given hazard, if any, is identified, and the consequences associated with the hazard are subsequently explored. Risk management is primarily a policy and decisionmaking process that uses risk assessment data in deciding how to avoid or mitigate the consequences identified in a risk assessment. Risk communication provides an important link between researchers, regulators, and the public through the sharing of risk analysis information and decisions with interested parties, including consumers.

Research Grants

USDA's Biotechnology Risk Assessment Research Grants Program focuses on areas of risk identified by Federal regulatory authorities through a consultation process. Program priority has been given to research aimed at:

- Assessing the risks of the loss of transgenes to wild and weedy populations.
- Exploring the potential for development of novel virulent plant viruses from genetic exchanges between viral transgenes and wild viruses
- Exploring the potential for indirect effects on the ecosystem of transgenic plants engineered to be insect resistant.

- Understanding the consequences of the genetic transformation of plant- and soil-associated microorganisms and the urgent need for relevant methodologies with which to study these effects.
- Understanding the genetic stability and behavioral changes of fish populations as a consequence of genetic modification.
- Assessing the risks of using recombinant fungi for biological control of weeds.
- Assessing the risks of using recombinant vaccinia virus for animal disease prevention.

Since 1992, when the program began, 180 proposals have been submitted for merit review, and 30 have been selected for funding. Proposals are evaluated for scientific quality and program relevance by peer panels composed of regulators and scientists familiar with risk assessment research.

Escape of Transgenes to Wild and Weedy Plant Ecosystems

Geneticists have warned that the primary negative environmental impact of crop biotechnology is likely to be the sexual transfer of engineered genes to related wild plants by natural hybridization, leading to the evolution of more aggressive weeds. Once engineered genes have spread into natural populations, containment may be difficult or impossible.

Dr. Norman C. Ellstrand of the University of California-Riverside is conducting a literature search to determine which of North America's 30 most important food crops are sexually fertile and typically co-occur with wild relatives in North America. This study is helping to identify the wild relatives that are sexually compatible with North American crops under cultivation. The report will state whether the resulting hybrids are vigorous, sexually fertile, and capable of asexual spread. Weeds that already have crossed with domesticated crop plants have been identified, and it will be determined whether those unions have led to the development of more aggressive weeds. The information culled from the literature search will help policymakers judge the potential among various crops for crop transgene escape and persistence in weedy populations.

Two sets of researchers—Drs. Jack Brown, Carol Mallory-Smith, and Donald Cecil Thill of the University of Idaho and Drs. C. Neal Stewart, Jr., Paul L. Raymer, and John N. All, of the University of Georgia—are investigating the fate and effects of gene transfer between domesticated canola (*Brassica napus*) and wild relatives, which are known to co-occur. Dr. Brown's team is evaluating the potential for the spread of genetically engineered herbicide resistance to wild, weedy relatives of canola in order to assess the potential for the emergence of new herbicide-resistant weeds. The investigators also are studying the potential for invasion of the herbicide-resistant plants into neighboring fields and are examining the overall impact of the introduction of transgenic herbicide-resistant canola into agricultural ecosystems.

A second canola study, conducted by Dr. Stewart and his colleagues, is focusing on the escape to the natural environment of canola plants that have been genetically engineered for insect resistance through introduction of the *Bacillus thuringiensis* (Bt) endotoxin gene *cryIA(c)*. The investigators have hypothesized that selection pressure from insect feeding, which is pervasive in natural environments, could enhance the fitness of the escaped transgenic canola, as it contains an insect resistance gene. The researchers will monitor vegetational changes over time on several field plots to assess selection pressure from lepidopteran insects feeding on the canola. The results should provide information on the short-term influence of escaped transgenic canola populations on natural plant communities.

In a study that was begun at Washington University and that will be completed at the University of Chicago, Dr. Joy M. Bergelson is determining the "physiological costs" of herbicide resistance relative to its benefits, as measured in terms of biological fitness. The project measures the effects of herbicide resistance in both a model weed system (*Arabidopsis thaliana)* and in an economically important crop system, canola. The first objective of the project is to determine how a gene conferring resistance to the herbicide chlorsulfuron influences the ability of *Arabidopsis* to invade natural communities. The rapid life cycle of *Arabidopsis* allows Dr. Bergelson to monitor population dynamics over several generations. The second objective is to measure the performance of transgenic canola with and without resistance to the herbicide.

Three other projects are focusing on other agricultural crops to determine the potential for transgene flow to other organisms. Drs. Daniel Z. Skinner and Richard N. Peaden of the Agricultural Research Service-Northern Plains Area are evaluating the risk that bees carrying alfalfa pollen will genetically contaminate alfalfa foundation seed-production fields in the vicinity of transgenic alfalfa. This research is determining the potential risks that bees, as pollen carriers, pose in terms of spreading transgenic alfalfa over both short and long distances.

Drs. Loren H. Rieseberg and Allison Snow are implementing a collaborative project involving Indiana University and The Ohio State University. The purpose of this project is to evaluate the frequency and consequences of gene flow between domesticated and wild sunflowers in agricultural production systems. Factors that affect gene escape, such as the role of physical distance, cultivar genotype, pollen competition, and hybrid fitness after successful hybridization, are being examined. The study is providing the first detailed empirical data on the frequency and consequences of gene flow between cultivated and wild sunflowers. This information will be helpful in establishing science-based guidelines to lessen the risks involved in the agricultural production of transgenic sunflowers. The data also will have implications for other outcrossing crop species with similar insect-pollinated reproductive systems.

Dr. Robert Hanneman, a scientist with the Midwest Research Area of the Agricultural Research Service, is identifying wild potato species that can form hybrids with the Irish potato. This information is essential for deciding how to introduce transgenic potato cultivars into areas in which native wild species of potato are found. The potato genome has a number of mechanisms that prevent fertilization, and these mechanisms need to be understood in order to predict the potential for hybridization between potato species. To acquire this information, scientists are examining hybrids between wild potato species and common cultivars. They will evaluate reproductive success through several generations of offspring and assess the rate at which transgenes are assimilated into native populations. This knowledge will be particularly useful in evaluating the risks of exporting transgenic potatoes to countries such as Mexico in which wild relatives of the Irish potato are commonly found.

References

Joy M. Bergelson. *Spread of Transgenic Weeds with Resistance to Herbicide*. Washington University, 1992.
Award: 36 months, $209,924

Jack Brown, Carol Mallory-Smith, and Donald C. Thill. *Gene Transfer Between Transgenic Canola (Brassica napus) and Related Weed Species*. University of Idaho, 1993.
Award: 36 months, $234,996

Norman C. Ellstrand. *Potential for Crop Transgene Escape and Persistence in Weedy Populations.* University of California-Riverside, 1994.
Award: 12 months, $31,981

Robert E. Hanneman. *Crossability Relationships Between Potato Varieties and Related Wild Species.* USDA Agricultural Research Service, Midwest Area , 1993.
Award: 36 months, $115,000

Loren H. Rieseberg and Allison Snow. *Gene Flow Between Domesticated and Wild Sunflowers.* Indiana University and The Ohio State University, 1993.
Award: 36 months, $180,000.

Daniel Z. Skinner and Richard N. Peaden. *Risk of Transgenic Alfalfa Dissemination During Seed Production.* USDA Agricultural Research Service, Northern Plains Area, 1993.
Award: 24 months, $72,000

C. Neal Stewart, Jr., Paul L. Raymer, and John N. All. *Bt Canola Under Selection: Genotype and Natural Plant Community Shifts.* University of Georgia, 1994.
Award: 48 months, $206,000

Risks of Genetically Engineering Plants To Be Virus Resistant

Introduction of certain fragments of a virus genome into plants can make the plants resistant to viruses that belong to the group from which the genome fragment was derived, or to viruses from a closely related group. Because few virus control strategies are presently available, genetically engineered plant resistance to viruses has received considerable attention in agricultural research. However, the scientific community is not in agreement about whether the use of virally transgenic plants could lead to creation of new viruses or to greater host ranges (i.e. a greater number of plant species that can be infected) for existing viruses. In the research projects detailed below, the risks of using this form of plant virus resistance are evaluated.

The types of insects that can transmit a virus from one plant to another are governed by the nature of the viral coat protein. Drs. Dennis Gonsalves and Mark Fuchs of Cornell University are studying transgenic plants producing the coat protein of a cucumber mosaic virus (CMV) isolate that is transmitted by aphids. They are evaluating the possibility that such plants could enable an infecting isolate of CMV, which is not normally aphid-transmissible, to be transmitted by aphids. This could occur if the nucleic acid of the infecting virus was by chance coated by the coat protein produced by the transgenic plant. The "transcoated" virus would not be expected to pass its aphid transmissibility on to subsequent generations of viruses. But this process could allow one generation of the virus to infect plants (via the aphid vectors) that would not otherwise be infected in the absence of the transgenic plant. The investigators are evaluating the risk of this type of virus spread under field conditions using transgenic tomatoes, squash, and melons.

Another set of projects is investigating two questions: Can the use of plants containing genetic material derived from viruses affect the frequency of recombination between viral ribonucleic acid (RNA, the genetic material of many viruses) sequences in plants? And can such transgenic plants alter the structure of the resulting recombinant viruses (i.e. create new viruses)?

It has been shown that when selective conditions favor survival of a recombinant virus, recombination can occur with other plant RNA viruses. It is not yet clear if, in the presence of transgenic plants, recombination can lead to the formation and survival of new viruses at a frequency greater than the natural background frequency of virus formation.

The research of Drs. William O. Dawson and Christopher M. Kearney of the University of Florida is designed to determine the basic frequency of recombination between virally derived genetic material in the plant and the genetic material of infecting viruses, with and without

strong selective conditions. The investigators are also measuring the frequency of recombination between different viral RNA's and between viral RNA and plant RNA in the absence of strong selective pressure. The results of this work will allow comparison between viral recombination frequencies in transgenic plants and in unmodified plants. The research is also designed to determine whether a naturally occurring virus could be stably incorporated as a viral transgene or as another portion of the plant genome to produce a novel, more virulent virus. In this study, recombination between numerous types of viruses is being examined, including tobacco mosaic viruses, tobamoviruses, potyviruses, and cucumoviruses.

Drs. Bryce Falk and George Bruening of the University of California-Davis are determining the frequency of recombination between viral RNA's that are very similar structurally and between viral RNA's that are very different structurally. They are determining the specific genetic structures necessary to permit recombination between viral RNA's. Detectable recombination events have been demonstrated in cases in which the two RNA's share moderate regions of nucleotide similarity. However, longer stretches of sequence similarity were required to detect recombination between viral RNA and a transgene derived from a virus than between two viral RNA's. Drs. Falk and Bruening are also investigating the effect of selective pressure on recombination frequency. The frequency of recombination between luteoviral RNA's and related RNA's is being examined using plant protoplasts (plant cells without walls) and transiently expressed viral gene sequences. With their protoplast system, the researchers are screening for recombinants of many combinations of viral sequences more quickly than they could in whole plants. This research is providing new tools for studying recombination events between virus genomes and virally derived genetic material in plants.

Dr. Zhongguo Xiong of the University of Arizona is studying infection of transgenic tobacco by red clover necrotic mosaic virus (RCNMV). He has developed an extremely sensitive and powerful assay that detects recombination between RCNMV RNA produced by the transgenic tobacco and RCNMV RNA from the infecting virus. In his system, only the recombinant virus produces systemic infection of the host plant. Direct evidence of recombination between an infecting virus and the transgenically expressed viral sequences has been observed, and in further experiments Dr. Xiong is characterizing the nature and origin of the recombination events using molecular markers both in the presence and in the absence of selective pressure. This information will help the investigators determine frequencies of transgenic recombination under a variety of conditions.

Research conducted by Drs. W. Allen Miller, P. John Rayapati, and Michael Lee at Iowa State University addresses two concerns relative to the release of transgenic oats expressing portions of the barley yellow dwarf virus (BYDV) genome that are involved in RNA replication. First, they are determining whether the virally derived transgene can assist or recombine with invading viral or subviral pathogens to increase the likelihood or severity of disease or to generate new virus strains. Second, they are assessing the risk of the escape of the viral gene by natural cross-pollination to wild, weedy relatives of domesticated oats.

In a related area, Dr. Vicki Vance of the University of South Carolina is investigating the consequences of multiple virus infections in a plant. Some plant diseases are caused by the interaction of two unrelated viruses infecting the same plant. This phenomenon is termed viral synergism. One risk of using transgenic plants containing virally derived genes is that a synergistic-type viral interaction could cause more severe viral disease in transgenic plants. This could lead to

higher levels of invading virus and thus might increase the extent of epidemics. The project is identifying the viral genes that are important in the induction of this response, using two model systems representing the major classes of plant viral synergisms. Dr. Vance is determining the smallest region of the viral genome acting as the "synergism gene," and intends to characterize the synergismat the molecular level.

At the Beltsville Agricultural Research Center of the Agricultural Research Service, Drs. Robert A. Owens, Rosemarie Hammond, and Theodor Diener are studying similar risk assessment questions, but with a different class of RNA particles called viroids. Viroids are the smallest autonomously replicating pathogenic agents yet described, and they are known for their destructiveness to a number of agricultural crops. Because no sources of naturally occurring genetic resistance to viroid-caused diseases are known, scientists are attempting to engineer durable resistance to the viroid disease through the expression of disabled particles inserted into the plant genome itself. Dr. Owens and his colleagues are evaluating the risks of this strategy by testing the ability of infectious viroids to recombine or otherwise support the replication and systemic spread of the disabled viroid within the plant.

References

William O. Dawson and Christopher M. Kearney. *Plant Viral Recombination Frequencies With and Without Selective Pressures: Basic Data for Estimating Risk of Virulent Strain Development from Viral Tendencies and Vectors.* University of Florida, 1994.
Award: 36 months, $223,000

Bryce W. Falk and George E. Bruening. *Risk Assessment: Recombination Between Virus Genomes and Genome Segments in Plants.* University of California-Davis, 1993.
Award: 36 months, $230,000

Mark Fuchs and Dennis Gonsalves. *Risk Assessment of Virus Spread Using Transgenic Plants Containing Viral Coat Protein Genes.* Cornell University, 1992.
Award: 24 months, $98,196

W. Allen Miller, P. John Rayapati, and Michael Lee. *Risks Associated with Transgenic Resistance to Barley Yellow Dwarf Virus.* Iowa State University, 1994.
Award: 36 months, $266,050

Robert A. Owens, Rosemarie W. Hammond, and Theodor O. Diener. *Stability of Disabled Viroid Sequences in Transgenic Plants: Generation of Wild-Type Viroids via Sequence Reversion and Recombination.* USDA Agricultural Research Service, Beltsville, 1992.
Award: 24 months, $56,200

Vicki B. Vance. *Identification of Plant Viral Synergism Genes.* University of South Carolina, 1993.
Award: 36 months, $130,000

Zhongguo Xiong. *Transgenic Recombination of RNA Viruses in Genetically Modified Plants.* University of Arizona, 1994.
Award: 36 months, $219,000

Risks to Ecosystem Stability of Plants Engineered To Be Insect Resistant

Controlling agricultural pests is like shooting bullets at a moving target: pests evolve so quickly that the control measures too often miss their target. The bacterium *Bacillus thuringiensis* (Bt) produces a class of substances that is toxic to many plant-eating insects. The Bt gene that produces this protein has been engineered into several crop plants as a means of providing plants with a pest defense mechanism, with the intention of reducing the amount of chemical pesticides that must be applied to a crop. However, there is a danger that careless use of this new technology could lead to a rapid development of resistance by insect pests to the genetically engineered Bt toxin.

Dr. David G. Heckel of Clemson University has designed a project to assess the risk of insect resistance development using the tobacco budworm and transgenic cotton containing the Bt gene. Dr. Heckel is evaluating three methods of assessing the risk that tobacco budworm would develop Bt resistance. This research is providing a solid base of information with which decisions can be made related to the management of pest-resistant crops expressing the Bt endotoxin.

A study by Drs. Joy M. Bergelson and Juliette Winterer at the University of Chicago is examining transgenic canola that contain genes for insect toxins derived from natural sources, including Bt endotoxin. These transgenic crops may pose indirect threats to the environment; when resistant pests evolve on these crops, the pests can endanger natural communities that rely on the same toxins to deter insect herbivores. The goal of the work is to develop a predictive evolutionary model that explores the effect of various resistance management tactics. The predictions of the model are being tested experimentally using canola that has been transformed with two natural toxin genes: a gene for proteinase inhibitor and a gene for Bt delta-endotoxin. The toxin-expressing canola is being planted in field enclosures into which the investigators will release diamondback moths, an important pest of canola. The rate at which the moth evolves resistance will then be monitored. The fate of resistant moths on wild plant relatives of canola is also being examined.

References

Joy M. Bergelson and Juliette Winterer. *Predicting the Spread of Insect Resistance: A Tool to Evaluate Ecological Risk.* University of Chicago, 1994.
Award: 36 months, $157,000

David G. Heckel. *Genetic Risk Assessment of Tobacco Budworm Resistance to Transgenic Bt-toxin.* Clemson University, 1992.
Award: 36 months, $230,000

Risks of Genetically Modified Plants and Microbes to Native Plant- and Soil-Associated Microbes

Surprisingly little is known about the ecology of microbes in soils of plant communities. This lack of information presents a challenge to the scientific community. Scientists see opportunities to apply the tools of biotechnology to modify microorganisms and thereby produce innovative solutions to agricultural and environmental problems. But scientists also recognize a need to gain an understanding of the environmental risks involved in applying biotechnological techniques to soil and plant microbes, and of the risks to soil and plant microbes involved in applying these techniques to plants.

Drs. Stephen K. Farrand and Ingyu Hwang of the University of Illinois are investigating the fate of environmentally released genetically modified microorganisms. Their study focuses on plasmid-mediated conjugation (direct transfer of genetic material between microbes), which is likely to be the most significant mechanism for gene exchange with other types of microbes. Experiments are specifically designed to assess the spread of genetic information between microorganisms that might typically come in contact with a genetically modified microorganism in a soil environment. By determining the factors that dictate whether or not a transferred trait will be retained by the recipient bacterium, Drs. Farrand and Hwang are attempting to characterize the potential risks of using recombinant organisms in agricultural and environmental applications.

Drs. Bradley J. Haack, Thomas E. Loynachan, and Robert E. Andrews of Iowa State University are also studying the potential for genetic exchange between soil bacteria mediated by bacterial conjugation. The researchers are evaluating the effects of soil conditions such as moisture, temperature, pH, and available nutrients on the conjugative process to better understand the conditions necessary for genetic exchange.

Drs. Christen D. Upper and Susan Hirano are studying the spread of bacteria on leaf surfaces of plants growing in patchy microenvironments. Differences in the suitability of plant microenvironments (e.g., roadsides or field margins) for growth of a particular bacterium present a challenge to the interpretation of risk assessment information. Very little is known about the adaptations of various bacteria to the patchiness of the agroecosystem. The results of these studies will help in understanding the factors, such as the type of bacteria and the substrate on which the bacteria are growing, contributing to the likelihood of bacterial spread. These studies are developing specific research methods and experimental protocols for assessing bacterial spread.

A microbe association with plants may also affect survival of the microbes in the soil. Drs. Eric W. Triplett and Sandra Austin-Phillips of the University of Wisconsin at Madison are studying the effects of genetically engineered alfalfa on the survival rate of nitrogen-fixing rhizobia bacteria of legumes. Three transgenic lines of alfalfa, which have been engineered to produce commercially important proteins, and a nonengineered control strain are being used to evaluate how gene additions to alfalfa affect microbial diversity. The number of beneficial microorganisms in the soil are being evaluated using sophisticated sampling techniques. Determining the ecological effects of these genetically engineered plants on microbial populations will be important in evaluating the risks associated with their use.

References

Stephen K. Farrand And Ingyu Hwang. *Assessing Horizontal Gene Transfer from Rhizosphere Microorganisms to a Genetically Modified Organism*. University of Illinois, 1992.
Award: 36 months, $220,000

Bradley J. Haack, Thomas E. Loynachan, And Robert E. Andrews. *Genetic Exchange Between Bacillus thuringiensis and Other Microbes in Soil*. Iowa State University, 1993.
Award: 36 months, $160,000

Eric W. Triplett and Sandra Austin-Phillips. *Effects of Transgenic Alfalfa on Soil Microbiology.* University of Wisconsin-Madison, 1994.
Award: 36 months, $122,122

Christen D. Upper And Susan Hirano. *The Impact of Patchiness and Life Strategy on Spread of Phyllosphere Bacteria*. University of Wisconsin-Madison, 1992.
Award: 24 months, $148,289

Risk Assessment Tools for Plant-Associated Bacteria

Fundamental to assessing the risk of genetically engineered bacteria are the tools needed to evaluate and measure populations of bacteria. Four projects have been funded to develop these tools.

Drs. Guy R. Knudsen and Louise-Marie Celine Dandurand of the University of Idaho are developing a model to predict dispersal and survival of bacteria applied as aerosols to crop plants. Microbial pest control agents are often applied using aerosol techniques, and risk assessment research is needed to evaluate the ways in which genetically engineered microbes applied in this manner may impact the environment through possible interactions with indigenous organisms. The model will describe optimal placement for sampling devices, determine probable detection thresholds for sampling, and create a system for predicting the effects of various weather conditions on dispersal and survival. The model is being developed from information obtained from experiments conducted under controlled conditions. In the experiments, selected strains of bacteria of diverse genera are being applied as aerosols to the foliage of bean plants. This research is creating a comprehensive model to predict bacterial survival and activity on plant foliage.

A project at the University of Wisconsin at Madison is using direct sampling of total bacterial DNA in the soil to obtain information on microorganisms in the plant root zone. Drs. Robert M. Goodman, Jo Handelsman, and Deborah A. Joseph are basing their work on the concept that microorganisms are sensitive indicators of environmental change because they have short life cycles, are physiologically diverse, and are omnipresent in ecosystems. They are developing better methods to allow for the assessment and description of soil microbial diversity and to apply these methods to monitor genetically engineered microorganisms in the environment. Field-grown transgenic potatoes are being used in the tests to evaluate the new methodologies for the detection of changes in the microfloral community.

Drs. Joseph Kloepper and Joe Shaw have created a *Pseudomonas flourescens* bacterium with the *lux* bioluminescent marker gene to test a novel system for monitoring the impact of bacteria under field conditions. The researchers are now comparing *lux* transformants with the wild-type strain in field plots. Their objective is to monitor the bioluminscence trait, to establish its genetic stability over several generations, and to determine whether bioluminescence is a useful tool for tracking bacteria. In additional studies, the impact of the transformed *Pseudomonas* on other bacterial species located on plant roots and in soil is being evaluated.

Drs. Silvia Rossbach and Frans J. de Bruijn of Michigan State University are developing a set of specific marker genes to create a genetic-based model system for monitoring a soil bacteria, particularly in root zones. Four DNA sequences involved in the regulation, transport, and degradation of a chemical substance called rhizopine have been identified. These catabolism genes are being used to monitor the survival and persistence of bacteria transformed with the genes and placed in the root zone of plants excreting rhizopine. This model system is allowing the evaluation of bacterial competition and fitness.

References

Robert M. Goodman, Jo Handelsman, and Deborah A. Joseph. *Does Expression of a Defense Transgene Alter Nontarget Rhizosphere Microflora?* University of Wisconsin-Madison, 1994.
Award: 24 months, $188,458

Joseph W. Kloepper and Joe J. Shaw. *Risk Assessment of a Plant-Associated Bacterium Genetically Engineered with Lux Genes.* Auburn University, 1993.
Award: 24 months, $137,000

Guy R. Knudsen and Louise-Marie C. Dandurand. *Model for Dispersal and Epiphytic Survival of Bacteria Applied to Crop Foliage.* University of Idaho, 1993.
Award: 24 months, $106,704

Silvia Rossbach and Frans J. de Bruijn. *Rhizopine Synthesis and Catabolism Genes for the Creation of Biased Rhizospheres.* Michigan State University, 1992.
Award: 36 months, $225,000

Risk Assessment of Aquatic Ecosystems

Transgenic fish (*e.g.*, catfish possessing a salmonid trout growth-hormone gene that promotes a 25-percent faster growth rate) have been produced using genetic engineering techniques. Because these transformed fish have an increased growth rate, they are of considerable interest to the commercial and sport-fishing industries. However, before these new, faster growing fish can be released into the environment, it is imperative that the risks they may pose to natural aquatic species be assessed, both in terms of competitiveness and in terms of gene escape to wild species.

Dr. Rex Dunham of Auburn University is focusing his research on the transgenic catfish and its potential risk to natural populations. Experiments are being undertaken to measure and evaluate the fitness of the transgenic catfish in terms of predator avoidance, foraging ability, and spawning ability relative to the fitness of nontransgenic catfish. The project intends to answer the question: Will transgenic fish with a trout growth-hormone gene be more competitive, and thus pose a risk to natural, competing catfish populations?

At Purdue University, Drs. William M. Muir, Richard D. Howard, and Christopher Bidwell are using medaka, a small, noncommercial fish native to rice fields, as a model organism to study the effects of gene insertion on anaquatic species measured over several generations. Although medaka is a noncommercial species, its short life cycle allows for multigenerational studies. The investigators are assessing the manner in which insertion of growth-hormone genes affects the competitive ability and reproductive success of the fish by evaluating and comparing transgenic and wild-type individuals under controlled conditions. They are also determining, over a period of eight generations, whether or not the expression of the transgene is changing, causing the transgenic fish to pose a greater environmental risk than was initially supposed.

References

Rex Dunham. *Predator Avoidance, Spawning, and Foraging Ability of Transgenic Catfish.* Auburn University, 1992.
Award: 36 months, $217,391

William M. Muir, Richard D. Howard, and Christopher A. Bidwell. *Adaptability and Genetic Stability of Transgenic Fish in Artificial Environments with Implications to Native Habitats.* Purdue University, 1993.
Award: 36 months, $177,656

Risks of Genetically Engineered Animal Vaccines

Dr. Tilahun D. Yilma of the University of California-Davis is conducting risk assessment studies on a recombinant vaccinia virus as a method to combat rinderpest disease of cloven-hoofed animals in East Africa, where rinderpest is endemic. The project is combining risk assessment studies with safety and efficacy trials to determine the stability of the vaccine, to determine whether or not the vaccine poses any danger to humans or other animals in the environment, and to assess the possibility that the virus might recombine with other viruses to form novel dangerous viruses. The results of this study could be applied to decisions about the release of other genetically engineered vaccines that are also based on the smallpox vaccine. Additionally, data from the study are expected to be helpful in the development of safety procedures for the use of new recombinant vaccines.

References

Tilahun D. Yilma. *Risk Assessment of a Vaccinia Virus Double Recombinant Vaccine for Rinderpest.* University of California at Davis, 1994.
Award: 36 months, $190,506

Risks of Genetically Enhanced Viral Biopesticides

Another type of biotechnology pest control, the use of genetically enhanced viral biopesticides, also is being considered as an alternative to traditional chemical pesticides. Naturally occurring baculoviruses kill pests but are not commercially successful as a means of crop pest control because up to 15 days are required to kill infected pests. Several baculoviruses have been engineered to contain toxin genes from a variety of sources that increase the rate at which the viruses kill the pests they infect. Before the baculoviruses are released for commercial use, however, an evaluation of their risks must be made to determine whether they pose a hazard to the environment. Drs. H. Allen Wood and Patrick R. Hughes of the Boyce Thompson Institute for Plant Research are assessing the environmental impact of genetically enhanced baculovirus pesticides to characterize the potential problems associated with their use as an alternative to chemical pesticides.

References

H. Alan Wood and Patrick R. Hughes. *Potential Interactions of Baculoviruses with Nontarget Insect Species*. Boyce Thompson Institute for Plant Research, 1994.
Award: 12 months, $93,730

Risks Associated With Using Fungal Plant Pathogens as Herbicides

At the University of Arkansas, Drs. David O. TeBeest and Cindy R. Cisar are exploring the risks associated with weed management that uses genetically engineered fungal plant pathogens as biological control agents. Fungal plant pathogens occur naturally, but they have a narrow host range and therefore are unsuitable for controlling the broad variety of different weed species that are commonly encountered in crop agriculture. Genetic engineering techniques have been proposed as a method to increase the host range and virulence of fungal plant pathogens, thus creating an effective bioherbicide. However, a careful assessment of the potential agricultural and environmental risks involved must be made before genetically enhanced bioherbicides are released into the environment. One major concern is whether the engineered genes of the fungi will be transferred between different strains of fungi via sexual reproduction, thus loosing mega-pathogens. In this project, the researchers are examining the mating ability of various naturally occurring strains of the fungus *Colletotrichum gloeosporioides* from a variety of plant hosts under simulated natural conditions.

References

David O. TeBeest and Cindy R. Cisar. *Sexual Cycle and Potential for Gene Flow in Fungal Biological Control Agents.* University of Arkansas, 1993.
Award: 24 months, $100,000

Biotechnology Risk Assessment Research Grants Program Annual Symposia

In 1993, The Biotechnology Risk Assessment Research Grants Program made a 3-year, $50,004 cooperative agreement with Maryland Biotechnology Institute scientists Drs. Morris Levin and J. Scott Angle. Under the agreement, these individuals are organizing and conducting annual risk assessment conferences associated with the USDA's Biotechnology Risk Assessment Research Grants Program.

The purpose of the conferences is to evaluate progress in risk assessment methodology and to identify missing scientific information needed by industry and agency regulators to conduct environmental risk assessment of genetically modified organisms. Scientists funded by the USDA's Biotechnology Risk Assessment Research Grants Program, the Environmental Protection Agency, and Environment Canada present reports and respond to questions about their research. Regulators make presentations on their roles in risk assessment and on the directions that regulation is taking in the area of biotechnology risk assessment.

The first annual conference, conducted jointly by USDA, EPA, and Environment Canada, was held June 22-24, 1994, at the University of Maryland in College Park, MD. Drs. David R. MacKenzie, Elizabeth Milewski, and Michael Goddard provided views on risk assessment from each of the three cosponsoring government agencies, and scientists funded by these agencies made presentations on their research. Dr. Rebecca Goldberg of the Environmental Defense Fund provided an environmentalist's perspective. A poster session was held. Dr. Jane Rissler of the Union of Concerned Scientists and Dr. Peter Day of Rutgers University made dinner presentations.

References

Morris Levin and Jay Scott Angle. *Proposal to Conduct Annual Risk Assessment Symposia.* Maryland Biotechnology Institute, 1993.
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